

# THERAPEUTIC USES FOR IP<sub>3</sub> RECEPTOR-MEDIATED CALCIUM CHANNEL MODULATORS

This application claims priority under 35 USC § 119(e) to Provisional  
5 Patent Application Serial Number 60/246,763, filed November 9, 2000.

## FIELD OF THE INVENTION

The present invention relates generally to the field of therapeutic  
compounds for preventing or ameliorating inflammation-related disorders, viral  
10 diseases, and some forms of cancer.

## BACKGROUND OF THE INVENTION

The transcription factor nuclear factor  $\kappa$ B (NF- $\kappa$ B) is a member of the  
NF- $\kappa$ B/Rel family which includes p50, p52, p65 (Rel A), c-Rel and Rel B proteins.  
15 Prototypical NF- $\kappa$ B is a p50-p65 heterodimer that is usually retained in the cytoplasm  
of unstimulated cells in an inactive form as part of a complex of inhibitory proteins  
(I $\kappa$ B). In response to various stimuli, the inhibitory protein is phosphorylated, rapidly  
ubiquitinated and subsequently proteolyzed by a 26 S proteasome complex. The  
degradation of I $\kappa$ B unmask the nuclear localization signal of the NF- $\kappa$ B heterodimer  
20 which then translocates into the nucleus where it binds to its cognate sequence to  
regulate gene transcription (Baeuerle and Baltimore, 1996, *Cell* 87:13-20; Mattson,  
1998, *Int. Rev. Neurobiol.* 42:103-168).

In the nervous system NF- $\kappa$ B is expressed in many cell types and is constitutively active in subsets of cells in rodent cortex and hippocampus (Kaltschmidt et al., 1994, *Mol. Cell. Biol.* **14**:3981-92). In neurons, NF- $\kappa$ B activity is elevated after seizure activity (Prasad et al., 1994 *Neurosci. Lett.* **170**:145-148; Rong and Baudry, 1996, *J. Neurochem.* **67**:662-668) global (Clemens et al., 1997, *Brain Res. Mol. Brain Res.* **48**:187-96) and focal (Schneider et al., 1999, *Nat. Med.* **5**:554-559) ischemia, and in Alzheimer's (Kaltschmidt, 1997, *Proc. Natl. Acad. Sci. USA* **94**:2642-2647; Akama et al., 1998, *Proc. Natl. Acad. Sci. USA* **95**:5795-5800) and Parkinson's diseases (Hunot et al., 1997, *Proc. Natl. Acad. Sci. USA* **94**:7531-7536). Because activation of NF- $\kappa$ B has been associated with cell injury and death in different pathological states, some investigators have proposed that this transcription factor contributes to the cell death process (Grilli et al., 1996, *Science* **274**:1383-1385; Clemens et al., 1997). However, results from cell culture and *in vivo* studies have demonstrated that activation of NF- $\kappa$ B represents a highly protective response in neurons (Barger et al., 1995, *Proc. Natl. Acad. Sci. USA* **92**:9328-9332; Mattson et al., 1997, *J. Neurosci. Res.* **49**:681-697; Taglialetela et al., 1997, *J. Neurosci. Res.* **47**:155-156; Yu et al., 1999, *J. Neurosci.* **19**:8856-8865). The neuroprotective role played by NF- $\kappa$ B involves the ability to induce the expression of genes encoding anti-oxidant (manganese superoxide dismutase) (Mattson et al., 1997), calcium stabilizing (Calbindin D28K) (Cheng et al., 1994, *Neuron* **12**:139-153), and anti-apoptotic proteins (Bcl-2) (Tamatani et al., 1999, *J. Biol. Chem.* **274**:8531-8538). A number of ligands have been shown to activate NF- $\kappa$ B, including tumor necrosis factor  $\alpha$  (TNF $\alpha$ )

(Barger et al., 1995; Hazan et al., 1990, *Proc. Natl. Acad. Sci. USA* **87**:7861-7865; Swingler et al., 1992, *AIDS Res. Hum. Retroviruses* **8**:487-493), IL1 $\alpha$  (Nonaka and Huang, 1990, *Mol. Cell. Biol.* **10**:6283-6289), glutamate (Kaltschmidt et al., 1995, *Proc. Natl. Acad. Sci. USA* **92**:9618-9622), nerve growth factor (Carter et al., 1996, *Science* **272**:542-545; Maggirwar et al., 1998, *J. Neurosci.* **18**:10356-10365), and secreted amyloid precursor protein (Barger and Harmon, 1997, *Nature* **388**:878-881). In addition, NF- $\kappa$ B is highly inducible by cellular stress: enhanced activity has been associated with elevated levels of oxidation, alterations in calcium homeostasis, and DNA damage (Mercurio and Manning, 1999, *Oncogene* **18**:6163-6171; Mattson et al., 2000, *J. Neurochem.* **74**:443-456; Gius et al., 1999, *Toxicol. Lett.* **106**:93-106).

In the central nervous system, changes in the concentration of intracellular calcium  $[Ca^{2+}]_i$  affect numerous functions including neurotransmitter release, and long-term potentiation and depression (Kennedy, 1989, *Trends Neurosci.* **12**:417-420; Berridge, 1998, *Neuron* **21**:13-26). However, the significance of  $Ca^{2+}$  released from internal stores such as endoplasmic reticulum (ER) has become increasingly apparent. In neurons, the ER is a continuous network of intracellular tubules and cisternae distributed throughout the cell and represents a large and releasable pool of intracellular calcium.

Regardless of its origin, increased  $[Ca^{2+}]_i$  often leads to the release of  $Ca^{2+}$  stored in the ER, a phenomenon referred to as calcium-dependent calcium release (Verkhratsky and Shmigol, 1996, *Cell Calcium* **19**:1-14). This phenomenon is regulated by the ER-resident IP<sub>3</sub> (inositol-1,4,5 triphosphate) and ryanodine receptor calcium channels. The intracellular signaling molecule IP<sub>3</sub>, the ligand for IP<sub>3</sub> receptors,

is generated by activation of phospholipase C and cleavage of phosphoinositol bisphosphate into diacylglycerol and IP<sub>3</sub>. Calcium regulates the sensitivity of these channels to IP<sub>3</sub>, increasing the probability that the channel will be open (Berridge, 1998). Therefore, alterations in [Ca<sup>2+</sup>]<sub>i</sub> will affect the activity of the ER IP<sub>3</sub> receptor, and thus affect cellular events regulated by this receptor. The phosphoinositide system is particularly well developed in the brain (Nahorski, 1988, *Trends Neurosci.* 11:444-448; Challiss et al., 1991, *Biochem. Soc. Trans.* 19:888-893; Furuichi and Mikoshiba, 1995, *J. Neurochem.* 64:953-960) and IP<sub>3</sub> receptors act as detectors that integrate information from neural signals (Berridge et al., 1998).

As will be appreciated by one knowledgeable in the art, the role and importance of calcium varies in different cell types. For example, as discussed herein, in neurons, there are calcium channels in the plasma membrane and, as discussed below, IP<sub>3</sub> receptors play a significant role. In cardiac cells, IP<sub>3</sub> is a minor player compared to the ryanodine receptor. Furthermore, in cardiac cells, calcium release is needed for contraction. It is of note that, as discussed below, there is no excitation-coupled release of calcium in immune cells.

Research into mechanisms of calcium signalling by the endoplasmic reticulum has thus followed two tracks: one involving excitation-contraction in muscle and the other calcium release by IP<sub>3</sub> and other cellular mediators. The specialized endoplasmic reticulum of muscle, the sarcoplasmic reticulum, has long been known to show the property of calcium-induced calcium release (Ebashi, 1991, *Ann. Rev. Physiol.* 53: 1-16; Schneider, 1994, *Ann. Rev. Physiol.* 56: 463-484). This finding led to the concept of 'trigger' calcium wherein a small increment of calcium triggers the

all-or-none calcium-induced calcium release process. In the case of heart muscle as well as other excitable cell types, it has been hypothesized that the trigger calcium comes from calcium influx through voltage-activated calcium channels as a result of the automatic, paced cardiac action potential (Putney and Ribeiro, 2000, *Cell. Mol. Life Sci.* **57**: 1272-1286).

It has also been hypothesized that depletion of stored  $\text{Ca}^{2+}$  may signal apoptosis (Bian et al., 1997, *Am. J. Physiol.* **272**: C1241-C1249), although others have reported that partial reduction of endoplasmic reticulum calcium stores actually protected against apoptosis (Pinton et al., 2000, *J. Cell Biol.* **148**: 857-862).

Inflammation represents a cascade of physiological and immunological reactions in response to non-recognized stimuli or pro-inflammation stimuli in an effort to localize or neutralize potentially harmful agents as well as prevent tissue damage. Inflammation involves the sequential release of various mediators including vasoactive mediators, chemoattractants, cytokines, prostaglandins, free radicals and proteases. Clinically, inflammation is a primary disease under acute conditions or is a manifestation of underlying pathophysiological abnormalities in chronic disease, characterized by classic signs of redness, pain, swelling and loss of function.

The transcription factor NF- $\kappa$ B is the major nuclear regulator of the inflammatory response in humans. NF- $\kappa$ B binding to specific sites on genomic DNA promotes the direct transcription of pro-inflammatory cytokine genes such as tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) and interleukins 1, 6, 8 and 12, chemokines, adhesion molecules, MMPs, Cox-2 and inducible nitric oxide (Tak and Firestein, 2001, *J. Clin. Invest.* **107**: 7-11). The cytokines in turn are the major stimulating force behind

inflammation. Of interest, pro-inflammatory cytokines, through binding to cell-surface receptors, also stimulate NF- $\kappa$ B activation, meaning that the inflammation cascade is a positive-feedback system.

The diseases or conditions that are associated with an inappropriate or  
 5 over-activation of the inflammation cascade include for example skin diseases such as psoriasis, autoimmune diseases, arthritis, atherosclerosis, chronic inflammatory demyelinating polyradiculoneuritis, Helobacter pylori-associated gastritis, inflammatory bowel disease (IBD), multiple sclerosis, asthma, lupus erythromatosis, systemic inflammatory response syndrome, Alzheimer's disease, stroke, meningitis,  
 10 allergies, toxic shock syndrome, anaphylactic shock, and many others.

Psoriasis is an inflammatory skin disease characterized by raised scaly lesions. Specifically, skin cells are pushed to the skin surface more quickly than the skin surface can shed dead skin cells. The end result is the formation of scaly lesions which are invaded by macrophage, lymphocytes and neutrophils, creating  
 15 inflammation and soreness of the tissue region. In addition, these cells may produce growth factors which may in fact cause skin cells to be produced even more rapidly, thereby worsening the condition. While the exact cause is unknown, psoriasis is hypothesized to be an autoimmune disorder.

Multiple sclerosis is an inflammatory disease that affects the nervous  
 20 system of an individual. Typically, the disease causes demyelination in the brain which in turn leads to a progressive loss of motor functions. While the cellular mechanism triggering destruction of the myelin is not understood, it is known that there is a localized increase in astrocyte proliferation and protease activity in afflicted

regions. As with psoriasis, the exact cause of multiple sclerosis is unknown although it is also hypothesized to be an autoimmune disorder.

Inflammatory bowel disease includes a number of specific diseases which cause intestinal inflammation or ulceration. For example, in ulcerative colitis, an inflammatory reaction involving the colonic mucosa leads to ulcerations. Furthermore, repeated inflammatory responses lead to fibrosis and a subsequent shortening of the colon. Similarly, Crohn's disease is characterized by chronic inflammation of all layers of the intestinal wall.

Arthritis is a chronic inflammatory disease which causes pain, swelling and destruction of joints and can also lead to organ damage. Specifically, the disease is characterized by infiltration of the synovial membrane with white blood cells and a thickening of the synovial membrane. There is subsequent tissue growth within the joints as well as the release of degrading enzymes and compounds associated with the inflammatory response which leads to progressive destruction of the cartilage tissue. It is of note that arthritis is also hypothesized to be an autoimmune disorder.

Asthma is characterized by recurring airway obstruction caused by inflammatory cell infiltration, smooth muscle cell proliferation and hypertrophy in the airway and mucus secretion into the airway lumen. While the exact etiology is not known, it is known that the condition involves infiltration and activation of inflammatory cells and that the inflammation is induced by cytokines.

Graft rejection occurs when the grafted tissue is recognized as foreign by the host's immune system. This rejection leads to inflammation and arteriosclerosis in the graft tissue and surrounding area, which in turn may lead to rejection of the

graft. Clearly, preventing activation of the inflammation cascade would prolong the life of the graft.

In addition to inflammation, NF- $\kappa$ B is also critically important in tumor growth. Many cancers have greatly increased NF- $\kappa$ B activity, which is part of the increased mitotic process, as well as a means of ensuring survival of the cells of the cancerous growth by inhibiting apoptosis. Specifically, abnormalities in the regulation of the NF- $\kappa$ B pathway are frequently seen in a variety of human malignancies including leukemias, lymphomas and solid tumors (Norris and Baldwin, 1999, *J. Biol. Chem.* **274**: 13841-13846). These abnormalities result in constitutively high levels of NF- $\kappa$ B in the nucleus of a variety of tumors, including breast, ovarian, prostate and colon cancers (Yamamoto and Gaynor, 2001, *J. Clin. Invest.* **107**: 135-142).

Activation of transcription factor NF- $\kappa$ B is also induced by a number of bacteria and viruses. The bacteria which induce NF- $\kappa$ B activity include, for example, EPEC, enteropathogenic *E. coli* (Savkovic et al., 1997, *Am. J. Physiol.* **273**:C1160-1167), *Gardnerella vaginalis* (Hashemi et al., 1999, *J. Infect. Dis.* **179**:924-930), *Helicobacter pylori* (Munzenmaier et al., 1997, *J. Immunol.* **159**:6140-6147), *Lactobacilli* (Klebanoff et al., 1999, *J. Infect. Dis.* **179**:653-660), *Listeria monocytogenes* (Hauf et al., 1994, *Infect. Immun.* **62**:2740-2747), *Mycoplasma fermentans* (Marie et al., 1999, *Infect. Immun.* **67**:688-693), *Mycobacteria tuberculosis* (Zhang et al., 1994, *Proc. Natl. Acad. Sci. USA* **91**:2225-2229), *Neisseria gonorrhoeae* (Naumann et al., 1997, *J. Exp. Med.* **186**:247-258), *Rickettsia rickettsii* (Sporn et al., 1997, *Infect. Immun.* **65**:2786-2791), *Salmonella dublin* (Eaves-Pyles et



al., 1999, *Infect. Immun.* **67**:800-804), *Salmonella typhimurium* (Hobbie et al., 1997, *J. Immunol.* **159**:5550-5559), *Shigella flexneri* (Dyer et al., 1993, *Infect. Immun.* **61**:4427-4433), *Staphylococcus aureus* (Busam et al., 1992, *Infect. Immun.* **60**:2008-2015). The viruses that induce NF- $\kappa$ B activity include for example, Human

5 Immunodeficiency Virus (HIV) (Bachelier et al., 1991, *Nature* **350**:709-712), Adenovirus (Shurman et al., 1989, *J. Immunol.* **143**:3806-3812), Epstein-Barr Virus (Hammariskjold and Simurda, 1992, *J. Virol.* **66**:6496-6501), Hepatitis B Virus (Siddiqui et al., 1989, *Virology* **169**:479-484), Cytomegalovirus (Sambucetti et al., 1989, *EMBO J.* **8**:4251-4258), HTLV-1 (Leung and Nabel, 1988, *Nature* **333**:776-778;

10 Ballard et al., 1988, *Science* **241**:1652-1655), Influenza Virus (Ronni et al., 1997, *J. Immunol.* **158**:2363-2374), Measles Virus (Harcourt et al., 1999, *J. Med. Virol.* **57**:9-16), Molony Murine Leukemia Virus (Pak and Fuller, 1996, *J. Virol.* **70**:4167-4172), Newcastle Disease Virus (Ten et al., 1992, *EMBO J.* **11**:195-203), Respiratory Syncytial Virus (Mastrorade et al., 1996, *J. Infect. Dis.* **174**:262-267; Garofalo et al.,

15 1996, *J. Virol.* **70**:8773-8781), Rhinovirus (Zhu et al., 1996, *J. Biol. Chem.* **271**:15815-15822; Zhu et al, 1996, *J. Clin. Invest.* **97**:421-430), Sendai paramyxo virus (Hiscott et al, 1989, *J. Virol.* **63**:2557-2566), Sindbis Virus (Lin et al., 1995, *J. Cell Biol.* **131**:1149-1161) and the Herpes Virus family, for example, Herpes Virus Saimiri (Yao et al., 1995, *Immunity* **3**:811-821), Human Herpes Virus 6 (Ensoli et al., 1989, *EMBO J* **8**:3019-3027), and Herpes Simplex Virus –1 (Gimble et al., 1988, *J. Virol.* **62**:4104-4112). A number of viruses have also evolved to include NF- $\kappa$ B binding sites within their promoters, for example, Hepatitis C Virus, Adenovirus (Williams et al., 1990, *EMBO J.* **9**:4435-4442), Avian Leukosis Virus (Bowers et al., 1996, *J. Virol.* **70**:3051-

3059), Bovine Leukemia Virus (Brooks et al., 1995, *J. Virol.* **69**:6005-6009), Cytomegalovirus (Sambucetti et al., 1989), Epstein-Barr Virus (Sugano et al., 1997, *J. Exp. Med.* **186**:731-737), Feline Leukemia Virus, HIV (Nabel and Baltimore, 1987, *Nature* **326**:711-713; Griffin et al., 1989, *Nature* **339**:70-73), Herpes simplex virus (Rong et al., 1992, *Virology* **189**:750-756), Polyoma virus (Ranganathan and Khalili, 1993, *Nuc. Acids Res.* **21**:1959-1964), Measles virus (Harcourt et al., 1999), Simian immunodeficiency virus (Bellas et al., 1993, *J. Virol.* **67**:2908-2913) and Simian Virus 40 (Kanno et al., 1989, *EMBO J.* **8**:4205-4214). Thus, those viruses which both induce NF- $\kappa$ B activation and have NF- $\kappa$ B binding sites within their transcription promoters would in essence be inducing their own replication upon transfection. It is also important to note that it has been hypothesized that a low level of NF- $\kappa$ B activation is part of the mechanism by which some viruses, for example, Epstein-Barr virus, Herpes simplex virus, Cytomegalovirus or Human Immunodeficiency virus maintain their chronic infections (Pahl, 1999, *Oncogene* **18**:6853-6866).

15           Infection by the Human Immunodeficiency virus, in most cases, leads to the development of acquired immunodeficiency syndrome (AIDS). Specifically, the HIV virus particle binds to CD4-bearing cells, such as T-lymphocytes, monocytes and macrophages, and is internalized through a viral-envelope mediated fusion of viral and host cell membranes. Therein, the virus relies on the interaction of cellular and virus-encoded trans-acting factors to produce the products necessary in order to assemble new virus particles. That is, the virus corrupts the cellular machinery of infected cells by causing the cell to replicate copies of the HIV virus. It is of note that cellular factors required by HIV include transcription factors NF- $\kappa$ B and SP1. The

infection leads to depletion of T-cells, which in turn causes immunodeficiency, leaving the host susceptible to secondary infections, which often prove fatal.

Initially, transcription of HIV is slow and inefficient, meaning that replication of the viral genome does not occur at a high rate. However, some of the transcripts are translated, producing viral proteins, one of which is Tat. Tat returns to the nucleus and binds to a structure formed in the viral RNA during transcription known as TAR. Tat binding at TAR is believed to activate transcription by recruiting transcription factors, thereby increasing the rate of transcription and Tat also promotes production of full-length viral transcripts. Several other roles have been proposed for Tat in addition to its role as a transcriptional activator. For example, it has been shown that infected cells shed or release tat during the acute phase of HIV infection. It has therefore been hypothesized that tat enters non-infected cells and disrupts host immune function by activating a wide variety of genes regulated by specific viral and endogenous cellular promoters (Vaishnav and Wong-Stall, 1991, *Ann. Rev. Biochem.* **60**: 577; Kumar et al., 1998, *J. Immunol.* **161**: 776). It has also been proposed that tat renders uninfected cells susceptible to productive viral infection (Goldstein, 1996, *Nature Medicine* **9**: 960-964). Furthermore, it has been proposed that tat acts on uninfected brain-derived cells to cause NF- $\kappa$ B activation and neurotoxicity as well as act directly on neurons to cause excitotoxicity and cell death by apoptosis (Chen et al., 1997, *J. Biol. Chem.* **272**: 22385-22388).

As discussed above, the activation of NF- $\kappa$ B is tightly regulated by immune-activation, cytokine-activation and stress-activation pathways in CD4<sup>+</sup> T cells and monocytes/macrophages, which are the cellular targets of HIV infection. Thus,

NF- $\kappa$ B has been hypothesized to play a role in HIV pathogenesis, although it has also been noted that the plethora of regulatory cascades suggest that while pharmacologic approaches may be developed to modulate NF- $\kappa$ B activity, the role that this modulation could play in the treatment of HIV infection is unclear (Rabson and Lin, 5 2000, *Adv. Pharmacology* 48: 161-207). However, these authors also noted that NF- $\kappa$ B can enhance HIV replication and can activate expression of HIV from latently infected cells, leading to the hypothesis that stimuli that induce NF- $\kappa$ B would lead to increased HIV replication and ultimately to a more rapid progression from asymptomatic HIV infection to AIDS.

10 PCT Application WO 99/02185 teaches the construction of a synthetic tat peptide and the use thereof for immunization against AIDS. It is also noted therein that tat protein is released extracellularly, making it available to be taken up by other infected cells to enhance transcription of HIV in the cells and to be taken up by non-infected cells, altering host cell gene activations and rendering the cells susceptible to 15 infection by the virus. Thus, tat uptake by both infected and uninfected cells is important for infectivity of HIV. Based on this, it is stated that immunization of mammals to induce antibodies to HIV tat protein could be used as a potential AIDS vaccine.

20 PCT Application WO 00/78969 teaches HIV-1-tat-multiple peptide conjugates and the use of same to induce an immune response. It is also noted therein that extracellular tat causes activation of intracellular signal transduction pathways that culminate in the production of various cytokines. Furthermore, it is proposed that one mechanism for viral activation by tat is the TAR-independent

activation of virus replication involving the host factor NF- $\kappa$ B by an intracellular signal transduction pathway.

US Patent 6,024,965 teaches the use of cytotoxic T-cell epitopes of the Rev and/or Tat protein for stimulating a specific cytotoxic T-cell response in a host.

5 This is based on the observation that the presence of cytotoxic T-cells to Rev and/or Tat in samples of a subject infected with HIV is an indication of a stable disease condition and a favourable prognosis of lack of progression to disease.

US Patent 5,821,046 teaches a synthetic RNA molecule arranged to bind tat protein. The use of the RNA molecule as a therapeutic for inhibiting HIV is also taught. Similarly, US Patent 5,637,461 teaches nucleic acid molecules for binding Tat protein.

US Patent 5,606,026 teaches the use of isolated natural IgM antibody in diagnosis of AIDS. The use of the low-affinity natural serum IgM to monitor the efficacy of therapeutic treatments is also taught. Furthermore, the idea that entry of Tat into resting, non-productive HIV infected cells activates these cells to produce virus is also disclosed.

A number of studies have shown that impairment of ER function with accumulation of proteins in the ER and the consequent release of  $\text{Ca}^{2+}$  from this organelle causes activation of NF- $\kappa$ B and NF- $\kappa$ B-dependent gene expression (Pahl and Baeuerle, 1995, *EMBO J.* **14**:2580-2588; Pahl and Baeuerle, 1997, *Trends Biochem. Sci.* **22**:63-7).

Xestospongic C (XeC) is a compound isolated from *Xestospongia* species. The use of XeC to block the inositol 1,4,5-triphosphate ( $\text{IP}_3$ ) receptor was

first described by Gafni et al. (Gafni et al., 1997, *Neuron* **19**:723-733). It has also been shown that XeC decreased the frequency of calcium oscillations in cardiac cells which was in turn linked to a parallel decrease in NF- $\kappa$ B activity (Hu et al., 1999, *J. Biol. Chem.* **274**:33995-33998). It was assumed that this established a direct link between

5 calcium oscillation frequency and the activity of NF- $\kappa$ B during agonist (histamine) stimulation. It is of note that this observation was held to be consistent with the prior art teachings, that is, that activation of NF- $\kappa$ B is calcium-dependent, as discussed above. It is also important to note that these experiments were carried out in cardiac cells, wherein the majority of the calcium within the endoplasmic reticulum of these

10 cells is regulated by the ryanodine receptor calcium channels, as the ryanodine receptors greatly outnumber the IP<sub>3</sub> receptors in cardiac cells. However, in other cell types, such as cells of the immune system and neurons, this is in fact reversed, with the vast majority of the calcium being regulated by the IP<sub>3</sub> receptors. Furthermore, the authors argued that calcium oscillation was important specifically in excitable cells

15 (i.e. neurons and muscle cells) due to the calcium link. It is of note that no mention is made of possible effects on immune cells, nor is it obvious at all that the regulation in cardiac cells would be equivalent, or even related, to regulation in immune cells, as discussed above. It is also of note that the concentration of XeC required to observe this effect in cardiac cells is very high, which suggests that the effect is not entirely

20 due to the IP<sub>3</sub> receptors but also due to binding of XeC at the ryanodine receptors.

In addition, therapies that target the IP<sub>3</sub> pathway have been proposed to be beneficial in the treatment of HIV dementia (Mayne et al., 2000, *J. Immunol.*

164:6538-6542), based on the observation that calcium release from IP<sub>3</sub> receptor regulated stores of calcium initiated tat-induced production of TNF- $\alpha$ .

As discussed above, NF- $\kappa$ B is the major nuclear regulator of the inflammatory response in humans. Calcium has previously been implicated as being a global NF- $\kappa$ B regulator, with Ca<sup>2+</sup> influx through the plasma membrane being widely held to be the main source of calcium-mediated signals. As will be appreciated by one knowledgeable in the art, the global importance of calcium levels predicts that pharmacological manipulation of calcium would be a poor choice for regulating NF- $\kappa$ B or for treating diseases and disorders characterized by NF- $\kappa$ B induction.

#### SUMMARY OF THE INVENTION

According to a first aspect of the invention, there is provided a pharmaceutical composition comprising an IP<sub>3</sub> receptor-mediated calcium channel blocker.

The IP<sub>3</sub> receptor-mediated calcium channel blocker may be a bis-1-oxaquinolizidine capable of blocking calcium release mediated by the IP<sub>3</sub> receptor.

The IP<sub>3</sub> receptor-mediated calcium channel blocker may be selected from the group consisting of: Xestospongin C; Xestospongin A; and Araguspongine B.

According to a second aspect of the invention, there is provided a method of treating HIV infection comprising:

administering an effective amount of a pharmaceutical composition comprising an IP<sub>3</sub> receptor-mediated calcium channel modulator to an individual in need of said treatment.

The IP<sub>3</sub> receptor-mediated calcium channel modulator may be selected from the group consisting of a PLC inhibitor; an IP<sub>3</sub> receptor-mediated calcium channel blocker; G-protein inhibitor; and mixtures thereof.

The G-protein inhibitor may be pertussis toxin.

5           The IP<sub>3</sub> receptor-mediated calcium channel blocker may be a bis-1-oxaquinolizidine capable of blocking calcium release mediated by the IP<sub>3</sub> receptor.

The IP<sub>3</sub> receptor-mediated calcium channel blocker may be selected from the group consisting of: Xestospongin C; Xestospongin A; and Araguspongine B.

10           According to a third aspect of the invention, there is provided a method of treating or preventing a disorder characterized by endoplasmic reticulum-dependent calcium release comprising:

          administering an effective amount of a pharmaceutical composition comprising an IP<sub>3</sub> receptor-mediated calcium channel blocker to an individual inflicted with the disorder characterized by endoplasmic reticulum-dependent calcium release.

15           The disorder characterized by endoplasmic reticulum-dependent calcium release may be selected from the group consisting of psoriasis, autoimmune diseases, inflammatory bowel diseases, pain, cardiac arrhythmia, hypertension, arthritis, multiple sclerosis, asthma, lupus erythromatosis, stroke, meningitis, allergies, toxic shock syndrome, anaphylactic shock, graft rejection, hypertrophic disease, viral  
20   diseases and uncontrolled growth diseases.

The viral disease may be selected from the group consisting of Adenovirus, Avian Leukosis Virus, Bovine Leukemia Virus, Cytomegalovirus, Epstein-Barr Virus, HIV, Hepatitis C Virus, Herpes simplex virus, Polyoma virus, Measles



virus, Simian immunodeficiency virus and Simian virus 40.

The uncontrolled growth disease may be cancer.

### BRIEF DESCRIPTION OF THE DRAWINGS

5                   FIGURE 1 shows the effects of XeC, Tg or vehicle on basal levels of NF- $\kappa$ B, NF- $\kappa$ B binding and one-week-old cultured cerebral cortical neurons from E-18 rat embryos. a) Neurons were treated with vehicle (C), 100 nM thapsigargin (Tg) or 1  $\mu$ M Xestospongin C (X). After 6 h, total cell extracts were prepared, and EMSA (electromobility shift assay) assays performed using oligonucleotides specific for NF- $\kappa$ B binding site. b) Autoradiographs from 4 separate EMSA assays were scanned and densitometric analyses were performed. Data are means  $\pm$  S.E.M. (\* $p$ <0.05 vs. control; Anova with Scheffe's post-hoc test).

10                   FIGURE 2 shows the results of experiments wherein following treatment of cells with either vehicle or 1  $\mu$ M XeC for 30 minutes cells were incubated with TNF $\alpha$ , Tg, or glutamate for 6 h, total cell extracts were prepared, and EMSAs were performed using oligonucleotides specific for NF- $\kappa$ B or CREB binding sites. a) effects of 100 ng/ml TNF $\alpha$  in the absence or presence of XeC on NF- $\kappa$ B binding. b) Films from 3 separate EMSAs from cells treated with vehicle (C), 100 ng/ml TNF $\alpha$  (T), or 1  $\mu$ M XeC for 30 min prior to TNF $\alpha$  (T + X) were scanned and densitometric analyses were performed. Data are means  $\pm$  S.E.M. \* $p$ <0.05 v. control, #  $p$ <0.01 v. TNF $\alpha$  alone; Anova with Scheffe's post-hoc test. c) Effects of 100 nM thapsigargin in the absence (Tg) or presence (Tg/X) of 1  $\mu$ M XeC on NF- $\kappa$ B binding. d) Effects of 20  $\mu$ M

glutamate in the absence (G) or presence (G/X) of 1  $\mu$ M XeC on NF- $\kappa$ B binding.

FIGURE 3 shows a) Diagrammatic representation of microsome and microsome extract (MSE) isolation technique. b) Equal amounts of protein from the subcellular fractions were separated by SDS-PAGE and immunoreacted with an antibody against an ER-resident protein Grp78. c) Supernatant fractions from brain cortex following 100,000 x g centrifugation for 1h (cytoplasmic fraction) were incubated 45 min with p50 antibody, p65 antibody, or a combination of p50 and p65 antibodies. Binding activity and band position were then analyzed using EMSA assay. The four sites of NF- $\kappa$ B binding identified are referred to as band A (A), band B (B), supershifted band 1 (ss1) and supershifted band 2 (ss2).

FIGURE 4 shows a) Aliquots of cytoplasmic fraction were left untreated (Cyt) or treated with 100 nM thapsigargin (Tg) or 1  $\mu$ M Xestospongine C (X) for 1h, and NF- $\kappa$ B binding activity was determined by EMSA. b) Aliquots of cytoplasmic fractions were left untreated (Cyt) or were treated with calcium at concentrations from  $10^{-6}$  to  $10^{-2}$  M for 1h, and NF- $\kappa$ B binding activity was determined by EMSA. c) Aliquots of cytoplasmic fraction was left untreated (Cyt) or treated for 1h with 10  $\mu$ M calcium ( $\text{Ca}^{2+}$ ), 10  $\mu$ M BAPTA-AM (B), or calcium and BAPTA-AM ( $\text{Ca}^{2+}$ /B), and NF- $\kappa$ B binding activity was determined.

FIGURE 5 shows a) Effects of microsomal extract on NF- $\kappa$ B binding activity. Microsomes were pretreated for 30 min with vehicle or 1  $\mu$ M XeC (X), and then incubated for 1h with vehicle (C), 100 nM thapsigargin (Tg), or 10  $\mu$ M calcium (Ca). The microsome suspension was then centrifuged at 100,000 g, and 3-5  $\mu$ l

microsome extract (MSE) were added to 8-10  $\mu$ l of cytoplasmic fraction. After incubation for 1h at 37 °C, NF- $\kappa$ B binding activity was determined. As a control, the cytoplasmic fraction without addition of microsomes (Cyt) was also analyzed. b) Exposures from 4 different experiments were scanned and densitometry was performed. Data are means  $\pm$  S.E.M. (\*p<0.05, \*\*p<0.01 vs. cytoplasm alone; +p<0.05 vs. thapsigargin alone; # p<0.05 vs Cyt/MSE, Anova with Scheffe's post-hoc test). c) Cytoplasmic fraction (Cyt), microsome extract (MSE), or pelleted microsomes (Pellet) were treated with vehicle (C), 1  $\mu$ M XeC (X), or 100 nM thapsigargin (Tg) for 1h prior to being analyzed for NF- $\kappa$ B binding activity.

FIGURE 6 shows the chemical structures of Xestospongin A, Xestospongin C and Araguspogine B.

FIGURE 7 shows bar graphs of p24 levels in peripheral blood lymphocytes 2 and 3 days post-infection with HIV CSF. CTRL cells were in media only, D1 cells were treated 1 time, 2 hours pre-infection with DMSO, D2 cells were treated once 2 hours pre-infection and once 6 hours post-infection with DMSO, X1 cells were treated 1 time, 2 hours pre-infection with XeC in DMSO and X2 cells were treated once 2 hours pre-infection and once 6 hours post-infection with XeC in DMSO.

FIGURE 8 shows bar graphs of p24 levels in cultures of peripheral blood lymphocytes 2, 3 and 4 days post-infection with HIV CSF. CTRL cells were in media only, D1 cells were treated 1 time, 2 hours pre-infection with DMSO, D2 cells were treated once 2 hours pre-infection and once 6 hours post-infection with DMSO, X1 cells were treated 1 time, 2 hours pre-infection with XeC in DMSO and X2 cells were treated once 2 hours pre-infection and once 6 hours post-infection with XeC in DMSO.

FIGURE 9 shows a graph of p24 levels in cultures of peripheral blood lymphocytes 2, 3 and 4 days post-infection with HIV CSF. CTRL cells were in media only, D1 cells were treated 1 time, 2 hours pre-infection with DMSO, D2 cells were treated once 2 hours pre-infection and once 6 hours post-infection with DMSO, X1 cells were treated 1 time, 2 hours pre-infection with XeC in DMSO and X2 cells were treated once 2 hours pre-infection and once 6 hours post-infection with XeC in DMSO.

FIGURE 10 shows bar graphs of p24 levels in cultures of peripheral blood lymphocytes 3, 4 and 5 days post-infection with HIV CSF. CTRL cells were in media only, D1 cells were treated 1 time, 2 hours pre-infection with DMSO, D2 cells were treated once 2 hours pre-infection and once 6 hours post-infection with DMSO, X1 cells were treated 1 time, 2 hours pre-infection with XeC in DMSO and X2 cells were treated once 2 hours pre-infection and once 6 hours post-infection with XeC in DMSO.

FIGURE 11 is a model of IP<sub>3</sub> receptor blocker in a calcium channel pore.

#### DESCRIPTION OF THE PREFERRED EMBODIMENTS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned hereunder are incorporated herein by reference.

## DEFINITIONS

As used herein, "effective amount" refers to the administration of an amount of a given compound that achieves the desired effect.

As used herein, "disease characterized by  $IP_3$  receptor mediated  
 5 release of calcium" refers to diseases or disorders which require  $IP_3$  receptor-mediated calcium release and subsequent NF- $\kappa$ B activation for disease progression. These include, but are in no way limited to, for example, psoriasis, autoimmune diseases, inflammatory bowel diseases, arthritis, cystic fibrosis, cachexia, muscle decay, multiple sclerosis, asthma, lupus erythematosus, stroke, meningitis, allergies,  
 10 toxic shock syndrome, anaphylactic shock, graft rejection, hypertrophic disease, viral diseases, for example, Adenovirus, Avian Leukosis Virus, Bovine Leukemia Virus, Cytomegalovirus, Epstein-Barr Virus, HIV, Hepatitis C virus, Herpes simplex virus, Polyoma virus, Measles virus, Simian immunodeficiency virus and Simian virus 40, and uncontrolled growth diseases, for example, some forms of cancer.

15 As used herein, "viral disease" refers to diseases or disorders caused by viruses that require NF- $\kappa$ B activation for replication. Examples include but are in no way limited to Adenovirus, Avian Leukosis Virus, Bovine Leukemia Virus, Cytomegalovirus, Epstein-Barr Virus, HIV, Hepatitis C virus, Herpes simplex virus, Polyoma virus, Measles virus, Simian immunodeficiency virus and Simian virus 40.

20 As used herein, "inflammation related disease" refers to diseases that are associated with an inappropriate or over-activation of the inflammation cascade. Examples of these type of diseases include but are by no means limited to arthritis, autoimmune diseases, inflammatory bowel disease, psoriasis, anaphylactic shock,

graft rejection, stroke, Alzheimer's disease, lupus erythromatosis, meningitis, multiple sclerosis, asthma, allergies, toxic shock syndrome and the like.

As used herein, "uncontrolled growth disease" refers to diseases characterized by high levels of NF- $\kappa$ B activation, for example, some forms of cancer.

5 As used herein, "XeC" refers to Xestospongins C, shown in Figure 6, a compound isolated from the *Xestospongia* species.

As used herein, "IP<sub>3</sub> receptor-mediated calcium channel blocker" refers to compounds capable of blocking calcium release from the endoplasmic reticulum mediated by the IP<sub>3</sub> receptor, for example, XeC, XeA, ArB, 2-APB, XeD, and DMXeB, as shown in Figure 6, or other compounds of the family bis-1-oxaquinolizidine capable of blocking calcium release mediated by the IP<sub>3</sub> receptor.

As used herein, "IP<sub>3</sub> receptor-mediated calcium channel modulator" refers to compounds capable of regulating IP<sub>3</sub> receptor-mediated calcium release, for example, IP<sub>3</sub> receptor-mediated calcium channel blockers, phospholipase C inhibitors, and some G protein inhibitors.

As used herein, "purified" does not require absolute purity but is instead intended as a relative definition. For example, purification of starting material or natural material to at least one order of magnitude, preferably two or three orders of magnitude is expressly contemplated as falling within the definition of "purified".

20 As used herein, the term "isolated" requires that the material be removed from its original environment.

As used herein, the term "treating" in its various grammatical forms refers to preventing, curing, reversing, attenuating, alleviating, minimizing,

suppressing or halting the deleterious effects of a disease state, disease progression, disease causative agent other abnormal condition.

Inflammation represents a cascade of physiological and immunological reactions. Clinically, inflammation is a disease when occurring chronically, and is characterized by redness, pain, swelling and loss of function. NF- $\kappa$ B is the major nuclear regulator of the inflammatory response in humans. As will be appreciated by one knowledgeable in the art, the global importance of calcium levels necessarily makes pharmacological manipulation of circulating and cytoplasmic calcium levels a poor choice for regulating NF- $\kappa$ B. However, it is shown herein that NF- $\kappa$ B activation can be inhibited using IP<sub>3</sub> receptor-mediated calcium channel modulators and that this inhibition occurs independent of calcium levels. This in turn indicates that IP<sub>3</sub> receptor-mediated calcium channel modulators can be used as therapies for treating inflammation-related disorders without significant side-effects, as would be the case if calcium levels were being altered.

As discussed herein, it is important to note that the prior art teaches that it is calcium crossing the plasma membrane and entering the cytoplasm of a cell that is required for activation of NF- $\kappa$ B within that cell. However, as discussed above, it is shown herein that NF- $\kappa$ B activation is independent of cytoplasmic calcium levels.

As discussed below, there are several areas for which blockage of IP<sub>3</sub> receptor-mediated calcium release may be therapeutic. For example, as a regulator of calcium waves, IP<sub>3</sub> receptor-mediated calcium channel modulators can be used to treat heart arrhythmia and fibrillation by regulating heart rhythm. Furthermore, as a regulator of NF- $\kappa$ B, it may be used for inhibiting inflammation related diseases,

uncontrolled growth diseases, and viral diseases, as discussed herein.

As discussed herein, treatment of cell cultures with an IP<sub>3</sub> receptor-mediated calcium channel modulator, for example, an IP<sub>3</sub> receptor-mediated calcium channel blocker, for example, XeC prior to and post HIV infection resulted in reduced virus levels (based on p24 levels) compared to control cultures. That treatment with an IP<sub>3</sub> receptor mediated calcium channel modulator is reducing the number of viral particles present in the supernatant clearly indicates that an IP<sub>3</sub> receptor mediated calcium channel modulator is a potential treatment for HIV. Specifically, the IP<sub>3</sub> receptor mediated calcium channel modulator is reducing viral load and viral particle assembly. This in turn indicates that an IP<sub>3</sub> receptor mediated calcium channel modulator on its own or in combination with other treatments known in the art and discussed herein can be used to treat HIV.

Although not wishing to be bound or limited to one particular theory, the inventor believes that extracellular tat is bound by cells at a receptor. Binding of tat protein at the receptor results in a G-protein-mediated activation of phospholipase C which in turn leads to production of IP<sub>3</sub>. IP<sub>3</sub> is bound by the IP<sub>3</sub> receptors on the ER which in turn leads to activation of NF-κB. In latent cells, induction of NF-κB leads to viral production; in uninfected cells, induction of NF-κB leads to induction of anti-stress and anti-apoptotic pathways. These pathways in effect prepare the uninfected cell for subsequent viral infection.

Thus, interfering with any stage of this pathway is a potential treatment for HIV. For example, phospholipase C inhibitors are well known in the art (see for example US Patent 5,847,074; US Patent 5,519,074; and US Patent 5,352,810) and



have been proposed for treating inflammation-related disorders and cancer. However, the use of these inhibitors for treating HIV has not been proposed or considered. Specifically, inhibiting phospholipase C would in turn prevent NF- $\kappa$ B activation which would reduce viral load and slow spread of the virus.

5 Furthermore, phospholipase C is known to be activated by G<sub>i</sub>/G<sub>o</sub> proteins and G<sub>q</sub> proteins. As such, inhibitors of these classes of G proteins, for example, pertussis toxin and the like, will also inhibit HIV disease progression.

10 In addition to blocking IP<sub>3</sub>-mediated calcium release using XeC and related compounds, it may also be possible to decrease HIV infectivity by lowering IP<sub>3</sub> receptor levels. It is of note that at least three isoforms of IP<sub>3</sub> have been identified and cloned (Furuichi et al, 1989, *Nature* **342**: 32-38; Mignery et al., 1990, *J. Biol. Chem.* **265**: 12678-126885; Sudhof et al., 1991, *EMBO J.* **10**: 3199-3205; Ross et al., 1992, *Proc. Natl. Acad. Sci. USA* **89**: 4265-4269; Maranto, 1994, *J. Biol. Chem.* **269**: 1222-1230; Yamada et al., 1994, *Biochem. J.* **302**: 781-790; Harnick et al., 1995, *J. Biol. Chem.* **270**: 2833-2840), meaning that gene replacement therapies and anti-sense probes directed against IP<sub>3</sub> receptors for treating HIV are within the knowledge of the art.

20 Other known inhibitors of IP<sub>3</sub> receptors include 2-aminoethoxydiphenyl borate (2-APB) (Gysembergh et al., 1999, *Am. J. Physiol.* **277**: H2458-H2469; Wilcox et al., 1998, *T.I.P.S.* **19**: 467-475), heparin (Ghosh et al., 1988, *J. Biol. Chem.* **253**: 11075-11079; Kobayashi et al., 1988, *Biochem. Biophys. Res. Comm.* **153**: 625-631) and a number of known monoclonal antibodies (Nakade et al., 1991, *Biochem. J.* **277**: 125-131; Sullivan et al., 1995, *Proc. Natl. Acad. Sci. USA* **92**: 8611-8615). While these

compounds are less than ideal due to non-specificity (heparin) and membrane impermeability, they may prove useful treatments for HIV provided these limitations can be overcome.

As will be appreciated by one knowledgeable in the art, combinations of  
5 the above-described treatments may also be used and are within the scope of the invention.

Thus, the IP<sub>3</sub> receptor mediated calcium channel modulators, for example, IP<sub>3</sub> receptor antibodies, IP<sub>3</sub> receptor antisense probes, heparin, PLC inhibitors, IP<sub>3</sub> receptor mediated calcium channel blockers, G protein inhibitors and  
10 the like discussed above act to limit IP<sub>3</sub> receptor mediated calcium release either by blocking release, blocking the receptor, or inhibiting receptor production. As will be apparent to one knowledgeable in the art, all of these may be used as treatments for HIV.

In some embodiments, the IP<sub>3</sub> receptor-mediated calcium channel  
15 blocker is Xestospongin C, shown in Figure 6 or a related compound. Xestospongin A, B, C and D represent a class of macrocyclic 1-oxaquinolizidines isolated from the Australian sponge, *Xestospongia exigua* (Nakagawa et al., 1984, *Tetrahedron Letters* **25**: 3227-3230). Subsequently, nine bis-oxaquinolizidine alkaloids (Araguspongines A-J) were isolated from *Xestospongin* (Kobayashi et al., 1989, *Chem. Pharm. Bull.* **37**:  
20 1676-1678). It has subsequently been shown that five of these compounds are potent blockers of IP<sub>3</sub>-mediated calcium release – XeA, XeC, XeD, ArB and demethylxestospongin B (DMXeB) (Gafni et al., 1997). Gafni et al. proposed that based on the lipophilic elongated core structure with two partially charged N groups

at either end (see Figures 6, 11), the listed compounds provide ideal lipophilic/hydrophilic moieties to fit into the IP<sub>3</sub> receptor channel port, as shown in Figure 11. As such, any drastic changes to net charge and molecular dimensions would likely have deleterious effects on the ability of the molecules to act as IP<sub>3</sub> receptor blockers.

Synthetically, Xestospongins A and other related alkaloids can be derived from bis-hydroxypyridinium dimer (Baldwin et al., 1998, *J. Am. Chem. Soc.* **120**: 8559-8560).

In other embodiments, the IP<sub>3</sub> receptor blocker may comprise a lipophilic elongated core separating two fused ring structures each containing a partially charged tertiary nitrogen. In some embodiments, the blocker is comprised of two oxaquinolizidine molecules joined by two carbon aliphatic chains. In this embodiment, each of the oxaquinolizidine structures formed by two fused rings can be in either the *cis* or *trans* configuration. Preferably, the rings are separated by 6 carbons and one ring is in the *cis* configuration while the other is in the *trans* configuration.

As will be appreciated by one knowledgeable in the art, a molecule having a length of 10-12 Angstroms, a lipophilic/hydrophilic (amphoteric) character to interact with the pore and a Van der Waals profile of approximately 8.22-9.38; 5.34-6.78; and 47.6-49.8 may act as an IP<sub>3</sub> receptor calcium channel blocker. Furthermore, the molecule may have a net charge between +1.5 and +2.0 at pH 7 and the bridgehead nitrogens may have a pKa around 11.

In yet other embodiments, the IP<sub>3</sub> receptor-mediated calcium channel

blocker is a molecule, for example, 2-APB (shown in Figure 6), which is structurally similar to a cleaved XeC molecule. That is, 2-APB has one ring portion and a side chain whereas XeC has two rings connected by side chains, as shown in Figure 6.

In some embodiments discussed below, the IP<sub>3</sub> receptor-mediated calcium channel modulator is used at a dosage or concentration of 0.1 to 100 μM. In other embodiments, the dosage may be 0.1 – 10 μM or 1 to 100 μM. In yet other embodiments, the dosage may be 1 to 10 μM.

As will be apparent to one knowledgeable in the art, a therapeutically effective amount of the IP<sub>3</sub> receptor-mediated calcium channel modulator is the amount sufficient to achieve the desired result. For example, for treating AIDS, the therapeutically effective amount is the amount sufficient to inhibit HIV replication and/or activation. It is well within the ability of a person skilled in the art to measure HIV activation and replication using well known markers such as T cell count, p24 assay, viral count etc. The amount administered will vary according to the concentration of the active agent and the body weight of the patient. Other factors include the degree of infection, the body weight and the age of the patient.

In some embodiments, the IP<sub>3</sub> receptor-mediated calcium channel modulator at concentrations or dosages discussed above may be combined with a pharmaceutically or pharmacologically acceptable carrier, excipient or diluent, either biodegradable or non-biodegradable. Exemplary examples of carriers include, but are by no means limited to, for example, poly(ethylene-vinyl acetate), copolymers of lactic acid and glycolic acid, poly(lactic acid), gelatin, collagen matrices, polysaccharides, poly(D,L lactide), poly(malic acid), poly(caprolactone), celluloses, albumin, starch,

casein, dextran, polyesters, ethanol, methacrylate, polyurethane, polyethylene, vinyl polymers, glycols, mixtures thereof and the like. Standard excipients include gelatin, casein, lecithin, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glyceryl monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, polyvinylpyrrolidone, sugars and starches. See, for example, Remington: The Science and Practice of Pharmacy, 2000, Gennaro, AR ed., Eaton, PA: Mack Publishing Co.

As will be apparent to one knowledgeable in the art, specific carriers and carrier combinations known in the art may be selected based on their properties and release characteristics in view of the intended use. Specifically, the carrier may be pH-sensitive, thermo-sensitive, thermo-gelling, arranged for sustained release or a quick burst. In some embodiments, carriers of different classes may be used in combination for multiple effects, for example, a quick burst followed by sustained release.

In other embodiments, an  $IP_3$  receptor-mediated calcium channel modulator at concentrations or dosages described above may be encapsulated for delivery. Specifically, the  $IP_3$  receptor-mediated calcium channel modulator may be

encapsulated in biodegradable microspheres, microcapsules, microparticles, or nanospheres. The delivery vehicles may be composed of, for example, hyaluronic acid, polyethylene glycol, poly(lactic acid), gelatin, poly(E-caprolactone), or a poly(lactic-glycolic) acid polymer. Combinations may also be used, as, for example, 5 gelatin nanospheres may be coated with a polymer of poly(lactic-glycolic) acid. As will be apparent to one knowledgeable in the art, these and other suitable delivery vehicles may be prepared according to protocols known in the art and utilized for delivery of the IP<sub>3</sub> receptor-mediated calcium channel modulator. Alternatively, the delivery vehicle may be suspended in saline and used as a nanospray for aerosol 10 dispersion onto an area of interest. Furthermore, the delivery vehicle may be dispersed in a gel or paste, thereby forming a nanopaste for coating a tissue or tissue portion.

It is of note that the IP<sub>3</sub> receptor-mediated calcium channel modulators as described above may be combined with permeation enhancers known in the art for 15 improving delivery. Examples of permeation enhancers include, but are by no means limited to those compounds described in U.S. Pat. Nos. 3,472,931; 3,527,864; 3,896,238; 3,903,256; 3,952,099; 4,046,886; 4,130,643; 4,130,667; 4,299,826; 4,335,115; 4,343,798; 4,379,454; 4,405,616; 4,746,515; 4,788,062; 4,820,720; 4,863,738; 4,863,970; and 5,378,730; British Pat. No. 1,011,949; and Idson, 1975, J. 20 Pharm. Sci. 64:901-924.

In some embodiments, the IP<sub>3</sub> receptor-mediated calcium channel modulator in any suitable form as described above, may be combined with biological or synthetic targeting molecules, for example, site-specific binding proteins,

antibodies, lectins or ligands, for targetting the IP<sub>3</sub> receptor-mediated calcium channel modulator to a specific region or location.

In other embodiments, the IP<sub>3</sub> receptor-mediated calcium channel modulator may be combined with other known treatments as a form of joint therapy.

- 5 For example, in the case of AIDS, the IP<sub>3</sub> receptor-mediated calcium channel modulator may be combined with other anti-HIV compounds, for example, azidothymidine (AZT), lamivudine (3TC), dideoxyinosine (ddi), dideoxycytidine (ddc) and ritonavir, as well as other reverse transcriptase and protease inhibitors.

As discussed above, the IP<sub>3</sub> receptor-mediated calcium channel  
 10 modulators inhibit activation of NF- $\kappa$ B which in turn inhibits activation of the inflammation cascade. As such, IP<sub>3</sub> receptor-mediated calcium channel modulators are suitable treatments for other disorders characterized by these processes, for example, disorders or diseases characterized by IP<sub>3</sub> receptor-mediated calcium releases.

15 As discussed above, asthma is characterized by recurring airway obstruction involving smooth muscle cell proliferation and inflammatory cell infiltration. Specifically, asthma is caused by swelling of bronchial tubes, often as a result of an allergic reaction. This swelling is substantially under the control of histamines and proinflammatory cytokines. Given that IP<sub>3</sub> receptor-mediated calcium channel  
 20 modulators in turn control cytokine production (as discussed above), these substrates would likely lessen the severity of asthma attacks. That is, the IP<sub>3</sub> receptor-mediated calcium channel modulator would accomplish one or more of the following: decrease the severity of or ameliorate symptoms, decrease the duration of attacks, increase the

frequency and duration of remission periods, prevent chronic progression of dyspnea, coughing and wheezing, improve hypoxia, increase forced expiration volume in one second, and improve resistance to airflow and hypocapnea/respiratory alkalosis. In embodiments for treating asthma, the IP<sub>3</sub> receptor-mediated calcium channel modulator may be arranged to be inhaled, for example, in a spray form, the preparation of which is described herein. It is of note that in this embodiment, the IP<sub>3</sub> receptor-mediated calcium channel modulator is in part acting as a smooth muscle relaxant and may be used in other instances wherein a smooth muscle cell relaxant is needed, for example, pupil dilation.

Cystic fibrosis causes the body to produce an abnormally thick, sticky mucus, due to the faulty transport of sodium and chloride (salt) within cells lining organs such as the lungs and pancreas, to their outer surfaces. The thick CF mucus also obstructs the pancreas, preventing enzymes from reaching the intestines to help break down and digest food. In addition, some patients develop progressive cytokine-mediated inflammatory lung disease. Given that IP<sub>3</sub> receptor-mediated calcium channel modulators in turn control cytokine production (as discussed above), these substrates would likely lessen the severity of CF (Ghio et al., 1996, *Am. J. Respir. Crit. Care Med.* **154**: 783-788). That is, the IP<sub>3</sub> receptor-mediated calcium channel modulator would accomplish one or more of the following: decrease the severity of or ameliorate symptoms, decrease the duration of attacks, increase the frequency and duration of remission periods, and decrease PMN-dominated airway inflammation (DiMango et al., 1998, *J. Clin. Invest.* **101**: 2598-2605; Tabary et al., 2000, *J. Immunol* **164**: 3377-3384). In embodiments for treating cystic fibrosis or other lung diseases in which NF-



κB has been implicated, for example, ARDS, systemic inflammation response syndrome, respiratory viral infections and occupational and environmental lung diseases (Christman et al, 2000, *Chest* **117**: 1482-1487), the IP<sub>3</sub> receptor-mediated calcium channel modulator may be arranged to be inhaled, for example, in a spray  
 5 form, the preparation of which is described herein.

Patients with chronic diseases such as cancer and AIDS often develop cachexia, a life-threatening disorder characterized by excessive weight loss and degeneration of skeletal muscle. Specifically, NF-κB in muscle cells suppresses the activity of MyoD, a transcription factor needed for muscle repair, by reducing the  
 10 levels of MyoD mRNA. Thus, inhibiting NF-κB would counter the MyoD inhibition (Guttridge et al., 2000, *Science* **289**: 2363-2365; Tisdale, 2000, *Science* **289**: 2293-2294; Lee et al., 2000, *Science* **289**: 2350-2354). That is, the IP<sub>3</sub> receptor-mediated calcium channel modulator would accomplish one or more of the following: decrease the severity of or ameliorate symptoms, decrease the duration of attacks, increase the  
 15 frequency and duration of remission periods, prevent muscle wasting and allow repair of damaged tissue.

Pain is a sensation resulting from tissue damage of threatened tissue damage. The IP<sub>3</sub> receptor mediated calcium channel modulator would be an effective treatment for pain by blocking calcium release along a nerve, thereby inhibiting  
 20 propagation of the signal. That is, the IP<sub>3</sub> receptor-mediated calcium channel modulator would accomplish one or more of the following: decrease the severity of or ameliorate symptoms, decrease the duration of attacks, and increase the frequency and duration of remission periods.

As discussed above, skin diseases, such as psoriasis, are characterized by rapid skin growth followed by inflammation and are generally characterized by hyperactivation of the immune system at the site of the disease on the skin. As discussed above, IP<sub>3</sub> receptor-mediated calcium channel modulators inhibit the inflammation cascade meaning that IP<sub>3</sub> receptor-mediated calcium channel modulators would be effective treatments for skin diseases. In these embodiments, the IP<sub>3</sub> receptor-mediated calcium channel modulators would be arranged for topical administration and may in some embodiments include permeation enhancers, as discussed above. In these embodiments, application of the IP<sub>3</sub> receptor-mediated calcium channel modulator to the afflicted area will inhibit rapid skin growth, thereby diminishing the severity of the symptoms. Specifically, the IP<sub>3</sub> receptor-mediated calcium channel modulator will accomplish at least one of the following: reduction in the number and/or size of skin lesions, lessening of cutaneous symptoms, for example, pain, burning and bleeding of the affected skin, inhibiting keratinocyte proliferation, and reducing skin inflammation.

As discussed above, multiple sclerosis is a chronic neurological disorder that affects the nervous system. Specifically, there is immune activation at the site of tissue destruction, suggesting that the immune system is involved in the destruction of the myelin. As discussed above, the IP<sub>3</sub> receptor-mediated calcium channel modulators have been shown to inhibit the inflammation cascade, meaning that the IP<sub>3</sub> receptor-mediated calcium channel modulators would be useful in preventing or slowing myelin destruction. That is, the IP<sub>3</sub> receptor-mediated calcium channel modulator would accomplish at least the following: decrease the severity of

symptoms, decrease the duration of disease exacerbations, increase the frequency and duration of disease remission and/or symptom free periods, prevent or attenuate chronic progression of the disease, improve visual symptoms, improve gait disorders, such as, weakness, axial instability, sensory loss, spasticity, hyperreflexia and/or loss of dexterity, improve cognitive impairment, reduce myelin loss, reduce breakdown of the blood-brain barrier and reduce perivascular infiltration of mononuclear cells. In these embodiments, the IP<sub>3</sub> receptor-mediated calcium channel modulator may be ingested as a tablet or pill, applied topically or injected, prepared at appropriate concentrations or dosages as described herein.

As discussed above, inflammatory bowel diseases are caused by intestinal inflammation and repeated inflammatory responses, meaning that inflammatory bowel diseases are hyper-inflammation diseases. As discussed above, given that the IP<sub>3</sub> receptor-mediated calcium channel modulators prevent NF- $\kappa$ B activation and cytokine production, the IP<sub>3</sub> receptor-mediated calcium channel modulators would also be an effective treatment for these disorders. That is, injection or infusion of the IP<sub>3</sub> receptor-mediated calcium channel modulators into the bowel or intestine will inhibit activation of the inflammatory system, thereby reducing the severity of the disease. Specifically, the IP<sub>3</sub> receptor-mediated calcium channel modulator would accomplish at least one of the following: decrease the frequency of the attacks, increase the duration of remission periods, decrease the severity or duration of abscess formation, intestinal obstruction, intestinal perforation and the like as well as ameliorate or reduce symptoms such as bloody diarrhea, abdominal pain, fever, weight loss and abdominal distension.

As discussed above, arthritis is believed to be an autoimmune disease, and is characterized by infiltration of the joints with inflammatory system cells. This causes swelling and edema in the delicate tissues of the joint, resulting in severe pain. In addition, immune cells release matrix metalloproteases and other destructive enzymes which break down the structure of the joint itself. Added to this is a decrease in functional blood flow caused by swelling, edema and vascular destruction. Thus, there is destruction of tissue and death of critical cells needed to repair bone and joint damage. This destruction is largely directed by  $\text{TNF}\alpha$  and other proinflammatory cytokines. As discussed above,  $\text{IP}_3$  receptor-mediated calcium channel modulators inhibit the inflammation cascade and also prevent  $\text{NF-}\kappa\text{B}$  activation in macrophages, indicating that these compounds would be an effective treatment for arthritis. Specifically, the  $\text{IP}_3$  receptor-mediated calcium channel modulator will accomplish at least one of the following: decrease severity of symptoms, including pain, swelling and tenderness of affected joints, weakness and fatigue, decrease severity of clinical signs, including thickening of the joint capsule, synovial hypertrophy, decreased range of motion, fixed joint deformity and soft tissue contractures, increase the frequency and duration of remission or disease-free periods and prevent or attenuate chronic progression of the disease. In these embodiments, the  $\text{IP}_3$  receptor-mediated calcium channel modulator is arranged to be injected directly into the afflicted joints or taken orally. Preparation of the  $\text{IP}_3$  receptor-mediated calcium channel modulators for injection is described herein.

Allograft tissue rejection has been shown to involve activation of  $\text{NF-}\kappa\text{B}$  and subsequent immune infiltration (Smiley et al., *Transplantation* **70**:415-419; Vos et

al., 2000, *FASEB J.* **14**:815-822). As discussed above, the IP<sub>3</sub> receptor-mediated calcium channel modulator prevents NF-κB activation and thereby induction of the inflammation cascade and would therefore act as a useful treatment for prevention of graft rejection. The IP<sub>3</sub> receptor-mediated calcium channel modulator will accomplish

5 at least one of the following: prolong the life of the graft; decrease the side effects associated with immunosuppressive therapy and decrease accelerated atherosclerosis associated with transplants. In other embodiments, a mesh coated or arranged to release the IP<sub>3</sub> receptor-mediated calcium channel modulators may be used in lieu of spraying the graft. Alternatively, the sprays or meshes could also be

10 used to treat, for example, venous leg ulcers, skin grafts, post-operative hypertrophy, hyperplasia, hypertrophic burn scars, hypertrophic gastropathy, cardiac hypertrophy associated with congestive heart failure and hypertrophic cardiopathy, or hypertension. The IP<sub>3</sub> receptor-mediated calcium channel modulator is also an effective treatment for cardiac cell death, as inhibition of NF-κB is anti-apoptotic and

15 IP<sub>3</sub> mediated calcium release is associated with diseased cardiac cells.

A stroke or cerebrovascular accident is caused by obstruction or rupturing of a cerebral vessel which in turn reduces the blood flow to the region of the brain supplied by the vessel. The reduced flow of blood results in an inadequate oxygen supply to the cells of the affected area and may cause permanent damage.

20 There are two major classes of stroke, termed ischemic and hemorrhagic. Ischemic stroke refers to a blockage of a blood vessel that leads to starving of the neurons of oxygen and glucose. Hemorrhagic stroke refers to a vessel bursting in the brain, leading to pressure from blood buildup, iron toxicity, and loss of oxygen and glucose

from those neurons that the vessel fed. Both of these conditions have inflammatory events as a portion of the pathology. Specifically, cytokines have associated toxicity and there is increased edema within the brain tissue itself. Furthermore, the majority of neuronal death caused by a mild or moderate stroke occurs within days to weeks after the initial insult. As discussed above, the IP<sub>3</sub> receptor-mediated calcium channel modulators inhibit the inflammation cascade, meaning that these compounds would be a useful treatment for stroke victims. Specifically, the IP<sub>3</sub> receptor-mediated calcium channel modulator will accomplish at least one of the following: increased blood flow, increased neuron survival, and improvement or amelioration of associated symptoms.

Cancer is essentially the result of uncontrolled division of cells that gives rise to abnormal growths or tumors and lack of apoptosis. When the tumors metastasize, the cancer can be spread throughout the body. As discussed above, NF- $\kappa$ B is important in cell division and is one of the most powerful anti-apoptotic responses of cells (Arya et al., 2000, *Surgery* **127**:366-369). In many cancers, the activity of NF- $\kappa$ B is high and has little or no feedback inhibition (Mayo and Baldwin, 2000, *Biochim. Biophys. Acta* **1470**:M55-62; Rayet and Gelinas, 1999, *Oncogene* **18**:6938-6947). As a consequence, cancer cells have more rapid and uncontrolled growth and are very resistant to stress. As discussed above, inhibiting NF- $\kappa$ B activation via the IP<sub>3</sub> receptor-mediated calcium channel modulators described herein would reduce the growth rate and make the cancer cells less resistant to stress and therefore more susceptible to treatments such as chemotherapy and radiation. Thus, the IP<sub>3</sub> receptor-mediated calcium channel modulators will accomplish at least one of

the following: reducing the growth rate of the cancer cells, making the cancer cells less resistant to other treatments, for example, radiation and/or chemotherapy and preventing spread of the cancer.

As discussed above, a number of viruses have also evolved to include NF- $\kappa$ B binding sites within their promoters, for example, Adenovirus, Avian Leukosis Virus, Bovine Leukemia Virus, Cytomegalovirus, Epstein-Barr Virus, Hepatitis C virus, HIV, Herpes simplex virus, Polyoma virus, Measles virus, Simian immunodeficiency virus and Simian virus 40. As discussed above, it has been hypothesized that a low level of NF- $\kappa$ B activation is part of the mechanism by which some viruses, for example, Epstein-Barr virus, Herpes simplex virus, Cytomegalovirus or Human Immunodeficiency virus maintain their chronic infections (Pahl, 1999, *Oncogene* 18:6853-6866). This means that inhibiting NF- $\kappa$ B activation using an IP<sub>3</sub> receptor mediated calcium channel modulator would inhibit replication of these viruses, and therefore would accomplish at least one of the following: prevent or limit viral particle assembly, prevent or decrease the rate of viral replication, decreasing viral load, prevent or limit the rate of viral infection and prevent further infection by the virus.

In addition, the HIV virus produces tat, which is in turn a powerful NF- $\kappa$ B activator. In the context of HIV infection, it appears that NF- $\kappa$ B has three major roles: 1) NF- $\kappa$ B is one of the most important transcription factors for production of HIV proteins, and is therefore critical for viral replication; 2) NF- $\kappa$ B activation, as discussed above, represents an anti-apoptotic response in cells, thus allowing the cells to remain viable during the infection and replication phase of the virus; and 3) NF- $\kappa$ B

promotes transcription of many pro-inflammatory cytokines, causing recruitment of surrounding immune cells, which may then become infected by virus particles exiting the host cell. Thus, activation of NF- $\kappa$ B represents a central strategy of HIV in order to aid in replication and to keep cells viable during the infection and replication stage.

5                   Previous work has shown that tat specifically causes release of calcium from IP<sub>3</sub> receptor mediated stores in the endoplasmic reticulum (Mayne et al., 2000). Thus, tat may represent a specific signal shed from HIV to activate NF- $\kappa$ B in surrounding cells in order to prepare them for infection. That is, as discussed herein, NF- $\kappa$ B activation enhances stress resistance in cells, meaning that infected cells are  
10 more likely to survive. In addition, even in the absence of tat, infected cells displaying increased NF- $\kappa$ B binding produce large amounts of pro-inflammatory cytokines which themselves cause NF- $\kappa$ B activation in neighbouring cells. Furthermore, astrocytes containing virus in a latent stage can be induced to become active viral producers by exposure to pro-inflammatory cytokines which activate NF- $\kappa$ B. However, IP<sub>3</sub> receptor-  
15 mediated calcium channel modulators will inhibit the ability of tat to activate IP<sub>3</sub> receptor-mediated calcium release in neurons and thus prevent NF- $\kappa$ B activation. In addition, human macrophages exposed to an IP<sub>3</sub> receptor-mediated calcium channel modulator were refractory to NF- $\kappa$ B activation and XeC treatment inhibited pro-inflammatory cytokine production in an immune cell line. As such, IP<sub>3</sub> receptor-  
20 mediated calcium channel modulator-mediated inhibition of NF- $\kappa$ B activation would accomplish at least one of the following: decrease viability of HIV infected cells, decrease the rate of viral replication, decrease the rate of further infection of the virus



and inhibit reactivation of viral replication in latent cells.

It is of note that IP<sub>3</sub> receptor-mediated calcium channel modulators could also be used as treatments for heart failure, cardiac myopathy, arrhythmia and hypertension. Specifically, an increase in IP<sub>3</sub> receptor-mediated calcium release is evident in diseased cardiac cells and there is evidence that this effect plays a role in the death of cardiomyocytes, and therefore in the risk of heart failure (Marks and Gutstein, 1997, *Heart Vessels Suppl.* **12**:53-57; Marks, 1997, *Am. J. Physiol.* **272**:H597-605). However, an IP<sub>3</sub> receptor-mediated calcium channel modulator, for example, XeC, would inhibit IP<sub>3</sub> receptor-mediated calcium release, thereby protecting diseased cardiac cells. IP<sub>3</sub>-mediated calcium release also plays a role in the rhythm of the heart (Jaconi et al., 2000, *Mol. Biol. Cell* **11**:1845-1858), meaning that XeC will also be a treatment for cardiac arrhythmia.

Hypertension, on the other hand, is thought to be caused by overactivation of the ER/SR calcium release pathway which in turn causes contraction of vascular muscles. This is thought to lead to hypertension as the vessels contract and give a smaller bore for blood to move through. As a consequence, the IP<sub>3</sub> receptor-mediated calcium channel modulators would relieve hypertension by reducing the release of ER/SR calcium and thereby relaxing smooth muscle.

The invention provides kits for carrying out the methods of the invention.

Accordingly, a variety of kits are provided. The kits may be used for any one or more of the following (and, accordingly, may contain instructions for any one or more of the following uses): treating: psoriasis, autoimmune diseases, inflammatory bowel diseases, arthritis, multiple sclerosis, asthma, cystic fibrosis, cachexia, lupus

erythromatosis, stroke, meningitis, allergies, toxic shock syndrome, anaphylactic shock, graft rejection, hypertrophic disease, hypertension, cardiac arrhythmia, pain, viral diseases, for example, Adenovirus, Avian Leukosis Virus, Bovine Leukemia Virus, Cytomegalovirus, Epstein-Barr Virus, Hepatitis C virus, HIV, Herpes simplex virus, Polyoma virus, Measles virus, Simian immunodeficiency virus and Simian virus 40, and uncontrolled growth diseases, for example, some forms of cancer or the like in an individual; preventing: an autoimmune response, spreading of a viral infection, swelling, pain, inflammation, prolonged inflammatory response or rapid cell or tissue growth in an individual at risk of: psoriasis, autoimmune diseases, inflammatory bowel diseases, arthritis, multiple sclerosis, asthma, cystic fibrosis, cachexia, lupus erythromatosis, stroke, meningitis, allergies, toxic shock syndrome, anaphylactic shock, graft rejection, hypertrophic disease, hypertension, cardiac arrhythmia, pain, viral diseases, for example, Adenovirus, Avian Leukosis Virus, Bovine Leukemia Virus, Cytomegalovirus, Epstein-Barr Virus, Hepatitis C virus, HIV, Herpes simplex virus, Polyoma virus, Measles virus, Simian immunodeficiency virus and Simian virus 40, and uncontrolled growth diseases, for example, some forms of cancer or the like in an individual; preventing one or more symptoms of: an autoimmune response, a viral infection, swelling, pain, inflammation, prolonged inflammatory response or rapid cell or tissue growth or the like in an individual at risk of: psoriasis, autoimmune diseases, inflammatory bowel diseases, arthritis, multiple sclerosis, asthma, cystic fibrosis, cachexia, lupus erythromatosis, stroke, meningitis, allergies, toxic shock syndrome, anaphylactic shock, graft rejection, hypertrophic disease, hypertension, cardiac arrhythmia, pain, viral diseases, for example, Adenovirus, Avian Leukosis

Virus, Bovine Leukemia Virus, Cytomegalovirus, Epstein-Barr Virus, Hepatitis C virus, HIV, Herpes simplex virus, Polyoma virus, Measles virus, Simian immunodeficiency virus and Simian virus 40, and uncontrolled growth diseases, for example, some forms of cancer or the like in an individual; reducing severity one or more symptoms of: an autoimmune response, a viral infection, swelling, pain, inflammation, prolonged inflammatory response or rapid cell or tissue growth in an individual; reducing recurrence of one or more symptoms of an autoimmune response, swelling, pain, inflammation, prolonged inflammatory response or rapid cell or tissue growth in an individual; suppressing: an autoimmune response, a viral infection, swelling, pain, inflammation, prolonged inflammatory response or rapid cell or tissue growth in an individual at risk of: psoriasis, autoimmune diseases, inflammatory bowel diseases, arthritis, multiple sclerosis, asthma, cystic fibrosis, cachexia, lupus erythromatosis, stroke, meningitis, allergies, toxic shock syndrome, anaphylactic shock, graft rejection, hypertrophic disease, hypertension, cardiac arrhythmia, pain, viral diseases, for example, Adenovirus, Avian Leukosis Virus, Bovine Leukemia Virus, Cytomegalovirus, Epstein-Barr Virus, Hepatitis C virus, HIV, Herpes simplex virus, Polyoma virus, Measles virus, Simian immunodeficiency virus and Simian virus 40, and uncontrolled growth diseases, for example, some forms of cancer or the like in an individual; delaying development of: an autoimmune response, viral infection, swelling, pain, inflammation, prolonged inflammatory response or rapid cell or tissue growth and/or a symptom of: psoriasis, autoimmune diseases, inflammatory bowel diseases, arthritis, multiple sclerosis, asthma, cystic fibrosis, cachexia, lupus erythromatosis, stroke, meningitis, allergies, toxic shock syndrome, anaphylactic

shock, graft rejection, hypertrophic disease, hypertension, cardiac arrhythmia, pain, viral diseases, for example, Adenovirus, Avian Leukosis Virus, Bovine Leukemia Virus, Cytomegalovirus, Epstein-Barr Virus, Hepatitis C virus, HIV, Herpes simplex virus, Polyoma virus, Measles virus, Simian immunodeficiency virus and Simian virus 40, and uncontrolled growth diseases, for example, some forms of cancer or the like in an individual; reducing duration of: an autoimmune response, a viral infection, swelling, pain, inflammation, prolonged inflammatory response or rapid cell or tissue growth in an individual.

The kits of the invention comprise one or more containers comprising an IP<sub>3</sub> receptor-mediated calcium channel modulator, a suitable excipient as described herein and a set of instructions, generally written instructions although electronic storage media (e.g., magnetic diskette or optical disk) containing instructions are also acceptable, relating to the use and dosage of the IP<sub>3</sub> receptor-mediated calcium channel modulator for the intended treatment, for example, psoriasis, autoimmune diseases, inflammatory bowel diseases, arthritis, multiple sclerosis, asthma, cystic fibrosis, cachexia, lupus erythromatosis, stroke, meningitis, allergies, toxic shock syndrome, anaphylactic shock, graft rejection, hypertrophic disease, hypertension, cardiac arrhythmia, pain, viral diseases, for example, Adenovirus, Avian Leukosis Virus, Bovine Leukemia Virus, Cytomegalovirus, Epstein-Barr Virus, Hepatitis C virus, HIV, Herpes simplex virus, Polyoma virus, Measles virus, Simian immunodeficiency virus and Simian virus 40, and uncontrolled growth diseases, for example, some forms of cancer or the like. The instructions included with the kit generally include information as to dosage, dosing schedule, and route of administration for the

intended treatment. The containers of the IP<sub>3</sub> receptor-mediated calcium channel modulator may be unit doses, bulk packages (e.g., multi-dose packages) or sub-unit doses.

5 The IP<sub>3</sub> receptor-mediated calcium channel modulator of the kit may be packaged in any convenient, appropriate packaging. For example, if the composition is a freeze-dried formulation, an ampoule with a resilient stopper is normally used, so that the drug may be easily reconstituted by injecting fluid through the resilient stopper. Ampoules with non-resilient, removable closures (e.g., sealed glass) or resilient stoppers are most conveniently used for injectable forms of the IP<sub>3</sub> receptor-mediated calcium channel modulator. Also, prefilled syringes may be used when the kit is supplied with a liquid formulation of the IP<sub>3</sub> receptor-mediated calcium channel modulator. The kit may contain the IP<sub>3</sub> receptor-mediated calcium channel modulator in an ointment for topical formulation in appropriate packaging. Also contemplated are packages for use in combination with a specific device, such as an inhaler, nasal administration device (e.g., an atomizer) or an infusion device such as a minipump.

As will be appreciated by one knowledgeable in the art, the IP<sub>3</sub> receptor-mediated calcium channel modulator may be combined or used in combination with other treatments known in the art when used or prepared to treat any of the above-referenced diseases.

20 The dose administered to a patient, in the context of the present invention should be sufficient to effect a beneficial therapeutic response in the patient over time. In determining the effective amount of the IP<sub>3</sub> receptor-mediated calcium channel modulator, the dose will be determined by the condition of the patient, as well

as body weight or surface area of the patient to be treated. Administration may be accomplished by a single dose or divided doses. For a typical 70 kg patient, a dose equivalent to approximately 0.1  $\mu$ g to 10 mg may be administered.

The invention will now be described further by way of examples.

5 However, it is important to note that the invention is not in any way restricted by these examples.

#### EXAMPLE I - NEURONAL CELL CULTURES

10 Cerebral cortices were removed from embryonic day 18 Sprague-Dawley rats (Harlan, Indianapolis, IN). Cells were dissociated as described previously (Mattson et al., 1995, *J. Neurochem.* **65**:1740-1751.) and were seeded into polyethyleneimine-coated 60 mm culture dishes containing Eagle's Minimum Essential Medium supplemented with 26 mM NaHCO<sub>3</sub>, 40 mM glucose, 20 mM KCl, 1 mM sodium pyruvate, 10% (v/v) heat-inactivated fetal bovine serum (Sigma, St. Louis, MO), and 0.001% gentamycin sulfate. After a 3-5 h incubation period to allow for cell attachment, the medium was replaced with 2 ml of Neurobasal Medium with B27 supplements (GIBCO Life Technologies, Grand Island, NY). Previous observations have shown that greater than 80% of the cells that attach in the first few hours after plating will survive longer than 7 days in Neurobasal Medium with no additional neurotrophic factors. Experimental treatments were performed on 7-to-9-day-old neuronal cultures in which there were approximately 5% contaminating glial cells.

20

#### EXAMPLE II - TOTAL CELL EXTRACT PREPARATION AND ELECTROPHORETIC

## MOBILITY SHIFT ASSAY

Total cell extracts were prepared as described (Baeuerle and Baltimore, 1988, *Cell* **53**:211-217). Briefly, cells were harvested, washed twice with ice-cold PBS and lysed for 30 min at 4°C in Totex buffer (20 mM HEPES, pH 7.9, 350 mM NaCl, 20% glycerol, 1% NP-40, 1mM MgCl<sub>2</sub>, 0.5 mM EDTA, 0.1 mM EGTA, 5 mM DTT, 0.1% PMSF, 1% aprotinin, 4 µg/ml leupeptin). Samples were centrifuged at 14,000 x g for 10 min and aliquots of supernatant were collected and stored at -70°C until taken for assay. The protein content of the extract (supernatant) was measured by Bradford method (BIO-RAD Laboratories, Hercules, CA, USA). For electromobility shift assays, equal amounts of protein were incubated in a 20 µl reaction mixture containing 20 µg BSA, 1 µg poly(dI-dC), 2 µl buffer D (20 mM HEPES, pH 7.9, 20% glycerol, 100 mM KCl, 0.5 mM EDTA, 0.25% NP-40, 2 mM DTT, 0.1% PMSF), 4 µl buffer F (20% Ficoll-400, 100 mM HEPES, pH 7.9, 300 mM KCl, 10 mM DTT, 0.1% PMSF), and 20,000-50,000 cpm of <sup>32</sup>P-labeled oligonucleotide (Promega, Madison, WI, USA) corresponding either to a NF-κB site (5'-AGT TGA GGG GAC TTT CCC AGG C-3') or a CREB binding site (5'-AGA GAT TGC CTG ACG TCA GAG AGC TAG-3').

After 20 min at room temperature, reaction products were separated on a 7% non-denaturing polyacrylamide gel. Competition experiments were performed by incubating extracts with labeled oligonucleotide probe in the presence of 100-fold molar excess unlabeled NF-κB or CREB oligonucleotide. To characterize the NF-κB complexes, supershift assays were performed. Extracts were preincubated for 45 min at room temperature with antibodies recognizing either p50 or p65 sub-units of NF-κB

(Santa Cruz Biotechnology, Santa Cruz, CA) or c-Fos (Oncogene Research Products, Cambridge, MA). The mixtures were further incubated with  $^{32}\text{P}$ -labeled probe and resolved as described above. Radioactivity of dried gels was detected by exposure to Kodak X-Omat film and images on the developed film were scanned into a computer  
 5 using a UMAX 1200s scanner. Densitometry was performed using Scion Image software (Scion Inc., Frederick, MD). Paint Shop Pro software (JASC Inc. Minneapolis, MN) was used for preparation of the final figures.

### 10 EXAMPLE III - PREPARATION OF CYTOPLASMIC AND MICROSOMES FRACTIONS

Cerebral cortices from adult female Sprague-Dawley rats were homogenized (20 strokes at 300 rpm) with a Teflon homogenizer in ice-cold buffer containing 1 mM EDTA, 0.32 M sucrose, 0.1 mM dithiothreitol and 1 mM HEPES as described previously (Chen et al., 1998, *Diabetes* 47:874-81) and cytoplasmic and  
 15 microsome fractions were isolated by differential centrifugation. Briefly, tissue fragments and cellular debris were removed by centrifugation at 500 g for 10 min and supernatants were centrifuged at 20,000 g for 20 min to pellet intact mitochondria and nuclei. The supernatant was then centrifuged at 100,000 g for 1h in order to obtain the cytoplasmic fraction and the microsomal pellet enriched in endoplasmic reticulum and  
 20 golgi membranes. Microsome extracts (MSE) were prepared by suspending microsomes in 1:100 v/v ice-cold buffer and centrifuging at 100,000 x g for 1hr. Microsomes were then re-suspended in 1:2 v/v volume buffer and treated as described below. At the end of the treatment, the suspension was centrifuged at



100,000 g for 1h, and supernatants were used as MSE. For experiments involving incubation of MSE with cytoplasmic extract, 3  $\mu$ l of MSE were added to 10  $\mu$ l cytoplasmic extract and mixtures were incubated at room temperature for 1h.

## 5 EXAMPLE IV - WESTERN BLOTTING

Separation and protein quantification of grp78 by western blotting was done using methods similar to those reported previously (Cheng et al., 1995, *J. Neurochem.* **65**:2525-2536.). Proteins in various cell fractions were separated by SDS-PAGE (10% acrylamide) using the method of Laemmli (Laemmli et al., 1970, *J. Mol. Biol.* **47**:69-85). Following electrophoretic transfer to nitrocellulose, the protein  
 10 was immunoreacted with a rabbit polyclonal antibody against grp78 (Stressgen Biotechnology) followed by incubation with peroxidase-labeled anti-rabbit IgG secondary antibody (1:4000). Immune blots were processed further using a chemiluminescence kit (Boehringer Mannheim) according to the manufacturer's  
 15 protocol. Images of blots were scanned and quantified as described above.

## EXAMPLE V - XESTOSPONGIN C DECREASES, AND THAPSIGARGIN INCREASES, LEVELS OF NF- $\kappa$ B ACTIVITY

The possible involvement of endoplasmic reticulum pools of intracellular  
 20 calcium regulated by IP<sub>3</sub> receptors in controlling basal levels of NF- $\kappa$ B was investigated using Xestospongine C (XeC) a specific membrane permeable modulator of IP<sub>3</sub> -mediated Ca<sup>2+</sup> release (Gafni et al., 1997, *Neuron* **19**:723-733; Hu et al., 1999, *J. Biol. Chem.* **274**:33995-33998), and thapsigargin (Tg), a specific inhibitor of ER

calcium/ATPase (Thastrup et al., 1990, *Proc. Natl. Acad. Sci. USA* **87**:2466-2470; Davidson and Varhol, 1995, *J. Biol. Chem.* **270**:11731-11734.). Cultured primary cortical neurons were treated with either 1  $\mu$ M XeC, 100 nM Tg or vehicle for 6h and protein extracts were analyzed for NF- $\kappa$ B binding activity by EMSA electrophoresis.

5 XeC caused a statistically significant ( $p < 0.05$ ) reduction in basal NF- $\kappa$ B binding activity relative to control levels, as shown in Figures 1a and 1b. Treatment with Tg, a compound that causes massive release of ER luminal calcium, produced a statistically significant ( $p < 0.05$ ) elevation in NF- $\kappa$ B activation as shown in Figures 1a and 1b. To demonstrate that the effects of XeC and Tg were specific for NF- $\kappa$ B, we  
 10 determined the levels of CREB, a calcium-sensitive transcription factor thought to play a role in learning and neuronal survival (Stevens, 1994, *Neuron* **13**:769-770; Huang and Stevens, 1998, *Essays Biochem.* **33**:165-178). In contrast to effects on NF- $\kappa$ B, XeC and Tg produced slightly augmented CREB binding activity; however, this was not statistically significant (data not shown).

#### 15 EXAMPLE VI - XEC INHIBITS INDUCIBLE LEVELS OF NF- $\kappa$ B IN CULTURED CORTICAL NEURONS

Because NF- $\kappa$ B is not only a constitutively active transcription factor, but is also highly inducible in neurons, we tested the ability of XeC to inhibit NF- $\kappa$ B  
 20 binding activity induced by the ligands TNF- $\alpha$  and glutamate. Cells were treated for 30 min with vehicle or XeC prior to being treated with vehicle (C) 100 ng/ml TNF $\alpha$  or 20  $\mu$ M glutamate for 6 h. As can be seen in Figures 2a and 2b, TNF $\alpha$  produced a

statistically significant ( $p < 0.05$ ) enhancement in NF- $\kappa$ B binding, although prior treatment with XeC completely abolished the ability of TNF $\alpha$  to activate NF- $\kappa$ B. As can be seen in Figure 2a, the effect appeared to be specific for NF- $\kappa$ B because neither TNF $\alpha$  nor XeC alone affected the binding activity of CREB. Induction of NF- $\kappa$ B binding activity was observed with Tg, and glutamate, and, as can be seen in Figures 2c and d, pretreatment with XeC completely abolished the elevation in NF- $\kappa$ B binding induced by these treatments. Cells pretreated with XeC prior to glutamate demonstrated NF- $\kappa$ B binding below that of control (vehicle-treated) neurons, again indicating that the decline in NF- $\kappa$ B binding with XeC was not directly due to reduced intracellular calcium.

#### EXAMPLE VII - MICROSOMES ARE ENRICHED IN GRP 78

Because XeC, a specific blocker of calcium release from IP<sub>3</sub>-receptor regulated pools, could abolish NF- $\kappa$ B activation induced by a disparate group of agents we conducted a series of cell-free experiments to determine mechanisms involved in this ER-mediated NF- $\kappa$ B activation. Microsomal preparations were isolated as described (Chen et al., 1998, *Diabetes* **47**:874-81) and illustrated (fig 3a) and tests conducted in order to determine the relative enrichment of endoplasmic reticulum membranes and functionality of ER-resident calcium channels. As shown in Figure 3b, western blots of Grp78, an ER-resident heat shock protein, performed on the various fractions demonstrated that the microsomal fraction was highly enriched in Grp78 and thus ER membranes.

## EXAMPLE VIII - CHARACTERIZATION OF NF- $\kappa$ B COMPLEXES IN CYTOPLASMIC FRACTION.

To determine the identity of the major NF- $\kappa$ B binding complexes found in our cytoplasmic fraction, supernatants removed following 100,000 x g centrifugation were incubated with p50 antibody, p65 antibody, or a combination of p50 and p65 antibodies for 45 min. The major bands observed in these experiments were identified as band A and band B, as shown in Figure 3c. Addition of p50 antibody shifted band A in its entirety to a much higher position, called ss1, and shifted the lower band to a slightly higher position, called ss2. Band A was also apparently diminished in intensity by p50 antibody treatment. Incubation with p65 antibody, as has been previously reported (Pahl and Baeuerle, 1995), caused a disappearance of band A, but had no apparent effect on band B. Incubation with p50 and p65 antibodies caused a complete shift upward of band A to the ss 1 position, shifted band B slightly upwards, and noticeably reduced band B. Thus, band A is the p50/p65 heterodimer while band B is likely the p50/p50 homodimer. In the following experiments, band A was generally the most abundant site of NF- $\kappa$ B binding, while the intensity of band B varied from barely detectable to the equal of band A.

## EXAMPLE IX - MICROSOMAL EXTRACT REGULATES CYTOPLASMIC NF- $\kappa$ B ACTIVITY IN AN ER-CALCIUM CHANNEL-DEPENDENT MANNER

Microsomal preparations were treated with thapsigargin and calcium in the absence or presence of XeC for 1h, centrifuged, and 3  $\mu$ l of supernatant (MSE)

were added to 10  $\mu$ l of the cytoplasmic fraction. After 1h at room temperature, samples were examined for NF- $\kappa$ B binding activity.

We then asked whether NF- $\kappa$ B could be induced by calcium directly. Aliquots of cytoplasmic fraction were treated with calcium at concentrations ranging from 1  $\mu$ M to 10 mM for 1h, followed by analysis of NF- $\kappa$ B binding by EMSA. As shown in Figure 4b, none of the calcium concentrations used increased NF- $\kappa$ B binding activity. These same extracts analyzed for CREB binding activity showed that calcium concentrations of 10  $\mu$ M, 100  $\mu$ M, and 1 mM greatly enhanced CREB binding, probably through CaM kinase activation. These data clearly demonstrate that while the elevation of calcium in cytoplasm by itself is able to induce CREB it is not sufficient to cause NF- $\kappa$ B activation. Conversely, it is also true that chelation of calcium does not produce a decline in binding activity. Aliquots of cytoplasmic fraction were treated for 1h with 10  $\mu$ M calcium, 10  $\mu$ M BAPTA-AM, or a combination of the two. As shown in Figure 4c, 10  $\mu$ M BAPTA-AM alone or in combination with calcium had no effect on the levels of the two major bands of NF- $\kappa$ B binding activity present in untreated cytoplasm.

#### EXAMPLE X – EFFECT OF XeC ON PERIPHERAL BLOOD LYMPHOCYTES

Peripheral blood lymphocytes, previously stimulated for 72 hours with PHA, were divided into 3 groups, designated T-HIV (RPMI-10 media containing 1  $\mu$ M XeC and 0.1% DMSO), N-HIV (RPMI-10 media containing 0.1% DMSO) and HIV (RPMI-10 media). The cells were then incubated in the dark at 37°C for 1 hour. The cells

were then infected with a macrophage-tropic CCR5-utilizing HIV CSF for 3 hours at 37° and then washed to remove excess virus. All groups were cultured in RPMI-10% FCS + 10U/ml IL-2. Referring to Figures 7-11, cells were incubated at 37°C in 5% CO<sub>2</sub>. At stated time points shown in the aforementioned figures, spent media was removed and replaced with fresh media. The media was tested for the presence of p24 antigen as a surrogate for HIV infection and replication. As can be seen from the data, there is inhibition of Xestospongin relative to DMSO. The significance of using the CCR5 utilizing macrophage-tropic virus JR-CSF is that this is the type of virus generally transmitted sexually.

Referring to Figure 7, the media-only control (CTRL) and the DMSO-treated cells (D1 and D2) show similar levels of p24 on both days 2 and 3. However, samples X2 (treated with XeC 2 hours pre-infection and 6 hours post-infection) and X1 (treated with XeC 2 hours pre-infection) show reduced levels of p24, approximately 80% and 50% of controls on day 2. However, the p24 levels of X1 and X2 increased to approximately control levels by day 3.

Referring to Figure 8, in a subsequent experiment, X2 samples showed greatly reduced p24 levels on days 2 and 3 while no effect was seen in the X1 samples. The kinetics of the data shown in Figure 8 can also be seen in Figure 9, wherein the levels of p24 in X2 is below the control levels on day 2 but increase to near wild-type levels by day 4.

Figure 10 summarizes data from a third experiment wherein p24 levels in X1 and X2 were both reduced relative to D1 and D2 on day 3. By day 4, p24 levels in X1 had increased to near control levels, but p24 levels in X2 samples remained

approximately 50% of the DMSO controls.

#### EXAMPLE XI - DISCUSSION

As discussed above, a wide range of conditions and ligands have been shown to activate the transcription factor NF- $\kappa$ B. Conditions that induce, and ligands that are associated with, cell stress responses including elevated levels of intracellular calcium, trophic factor withdrawal, oxidative stress and exposure to ultraviolet light as well as activation of cell surface receptors by cytokines such as TNF- $\alpha$  and interleukins have been shown to enhance the binding and/or transcriptional activity of NF- $\kappa$ B (Pahl, 1999, *Oncogene* **18**:6853-6866). Some have suggested that increased levels of intracellular calcium may be an underlying factor common to such activation of NF- $\kappa$ B (Ginn-Pease and Whisler, 1998). Specifically, it has been suggested that release of intracellular stores of calcium may play a major role in activation of NF- $\kappa$ B (Pahl and Baeuerle, 1996, *FEBS Lett.* **392**:129-136; Quinlan et al., 1999, *J. Immunol.* **163**:5656-5665; Sen et al., 1996, *FEBS Lett.* **385**:58-62.). To help elucidate the relationship between ER calcium release, through IP<sub>3</sub> receptors specifically, and NF- $\kappa$ B activation we conducted a series of experiments and found that a) XeC reduced both basal and inducible NF- $\kappa$ B binding activity in cortical neurons, and b) contrary to the teachings of the prior art, this decline was independent of changes in intracellular calcium. Thus our data indicates that it is not the calcium release from ER stores that activates cytoplasmic NF- $\kappa$ B, but rather the decrease in intraluminal calcium.

We have demonstrated that a dose of XeC reported to be highly specific

for blocking ER IP<sub>3</sub> calcium channels (Gafni et al., 1997, Hu et al 1999) can inhibit basal levels of NF-κB binding. XeC also significantly inhibited the ability of TNFα to induce NF-κB. Glutamate, which causes a large influx of calcium in neurons, also greatly induced NF-κB binding activity. XeC was able to totally abolish this induction, although XeC treatment did not erase the increase in [Ca<sup>2+</sup>]<sub>i</sub> caused by glutamate (data not shown). Glutamate induces activation of IP<sub>3</sub> receptors both by increasing intracellular calcium (Kato and Rubel, 1999, *J. Neurophysiol.* **81**:1587-1596; Nakamura et al., 1999, *Neuron* **24**:727-737) and by induction of IP<sub>3</sub> production through phospholipase C (PLC) (Liu et al., 1997, *Eur. J. Pharmacol.* **338**:277-287; Recasens and Vignes, 1995, *Ann. N. Y. Acad. Sci.* **757**:418-29). Therefore, all inducers of NFκB tested here have in common the ability to initiate calcium release through ER IP<sub>3</sub> receptors, either through increasing intracellular calcium, activating production of IP<sub>3</sub>, or both. In the case of glutamate, an increase in intracellular calcium was not able to activate NFκB when IP<sub>3</sub> receptors were blocked with XeC.

The fact that neither calcium nor BAPTA-AM were able to change NFκB binding activity shows that the calcium released from the microsomes does not regulate NFκB. Indeed, the role calcium plays in this paradigm seems to be as an intraluminal signal. This is supported by the observation that direct activation of IP<sub>3</sub> receptors on microsomes using IP<sub>3</sub> itself led to MSE which had NF-κB stimulating properties similar to that seen with Tg. In addition, pretreatment of microsomes with XeC completely abolished this effect. Microsomes pretreated with XeC followed by calcium produced no NF-κB stimulating activity, indicating that though calcium can



directly modulate ER calcium release, it has no effect when IP<sub>3</sub> receptors are blocked.

As discussed above, many studies implicate calcium as having a central role in governing NF- $\kappa$ B activity. We have shown in our *in vitro* studies that calcium per se did not affect NF- $\kappa$ B binding. However, our cell-free system only examined the

5 NF- $\kappa$ B signaling pathway from the ER to NF- $\kappa$ B activation. Indeed, in the whole cell, our hypothesis that it is the filling state of the ER IP<sub>3</sub> pool which signals NF $\kappa$ B activation would tend to implicate calcium as a major upstream effector. However, both IP<sub>3</sub> and ryanodine receptors are acutely sensitive to [Ca<sup>2+</sup>]<sub>i</sub>, as is the ER resident calcium ATPase. In addition, IP<sub>3</sub> synthesis is sensitive to calcium levels (del Rio et

10 al., 1994, *J. Neurochem.* **63**:535-43; Kim et al., 1999, *J. Biol. Chem.* **274**:26127-26134). In fact, in the absence of calcium, PLC-mediated production of IP<sub>3</sub> drops precipitously (Hughes and Putney, 1990, *Environ. Health Perspect.* **84**:141-147). As ER IP<sub>3</sub> receptors are primarily regulated by IP<sub>3</sub> and calcium, additional of calcium chelators will greatly reduce the activity of IP<sub>3</sub> receptors due to the fact that both

15 mediators of channel opening are diminished. In addition, BAPTA-AM specifically interferes with IP<sub>3</sub> binding to the receptor (Taylor and Broad, 1998, *Trends Pharmacol. Sci.* **19**:370-375) and this information taken as a whole could explain a great deal of what has been observed regarding calcium regulation of NF- $\kappa$ B binding. The ER-overload response, examined extensively by Pahl et al., has been shown to

20 require release of calcium from ER. Therefore, many of the most commonly used activators of NF- $\kappa$ B impinge upon the endoplasmic reticulum, through elevations in intracellular calcium, activation of PLC to produce IP<sub>3</sub>, or both.

NF- $\kappa$ B is a critical mediator of immune and inflammatory responses in a variety of peripheral cells where it has been shown to enhance the transcription of many pro-inflammatory cytokines (Karin and Delhase, 2000, *Semin. Immunol.* **12**:85-98; Akira and Kishimoto, 1997, *Adv. Immunol.* **65**:1-46). Furthermore, activation of

5 NF- $\kappa$ B has been implicated in a large and growing number of physiological events, many of them associated with exacerbation of pathological states. For example, overactivation of NF- $\kappa$ B is associated with autoimmune and inflammatory diseases, such as ulcerative colitis (Neurath and Pettersson, 1997, *Immunobiology* **198**:91-98),

10 asthma (Ray et al., 1995, *Chest* **107**:139S), multiple sclerosis (Hilliard et al., 1999, *J. Immunol.* **163**:2937-2943) and arthritis (Seetharaman et al., 1999, *J. Immunol.* **163**:1577-1583), as well as HIV infection (Swingler et al., 1994, *Biochem. Biophys. Res. Commun.* **203**:623-630), and cancer (Foo and Nolan, 1999 *Trends Genet.* **15**:229-235). Therefore, whereas discovering specific and potent means of activating

15 NF- $\kappa$ B in neurons may lead to novel neuroprotective strategies, discovering specific means of decreasing NF- $\kappa$ B activation in other cell types may lead to treatments for inflammatory diseases and cancer.

While the preferred embodiments of the invention have been described above, it will be recognized and understood that various modifications may be made therein, and the appended claims are intended to cover all such modifications which

20 may fall within the spirit and scope of the invention.